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Hindsight and Rapid Escape in a Freshwater Oligochaete

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Abstract. A novel escape reflex involving the posterior end of a freshwater oligochaete worm, *Lumbriculus variegatus*, is described. Electrophysiological recordings and videotape analysis from submersed, freely behaving worms show that either a moving shadow or sudden decrease in light intensity evokes repetitive spiking in lateral giant nerve fibers (LGFs) and rapid tail withdrawal when the worm's posterior end is positioned at the air-water interface, to facilitate gas exchange. Because comparable electrical and behavioral response patterns occur in isolated posterior body fragments, but not in midbody or anterior fragments, we conclude that the LGF shadow-sensitivity is localized in posterior segments. Added support for this idea is provided by electron microscopic observations demonstrating the presence of candidate photoreceptor cells in the epidermis of posterior segments. These cells are invaginated distally to form a cavity (phaosome) filled with microvilli, and resemble the known photoreceptors in anterior segments of earthworms and leeches.

Introduction

Rapid escape responses to stimulus modalities such as touch and substrate vibration are widespread in polychaete and oligochaete worms (Mill, 1975, 1978; Dorsett, 1978, 1980; Drewes, 1984). However, relatively few annelid species exhibit rapid escape responses to photic stimulation (Steven, 1963; Mill, 1978), and these have not been extensively studied.

Examples of polychaetes with photically activated escape responses include several species of nereids (Clark, 1960; Evans, 1969, Gwilliam, 1969), sabellids, and most serpulids (Nicol, 1948, 1950; Krasne, 1965). In all of these groups, key features of the responses are: (1) adequacy of abrupt decreases, but usually not increases, in light intensity for eliciting escape; (2) localization of photosensitivity into anterior segments; (3) rapid withdrawal of the worm's anterior end or branchial crown which is often modified for respiration as well as feeding; (4) apparent mediation of escape by giant nerve fibers; and (5) rapid habituation with repeated stimuli.

Although rapid escape reactions to mechanosensory modalities exist in many species of terrestrial and freshwater oligochaetes (Drewes *et al.*, 1983; Drewes, 1984; Zoran and Drewes, 1987), escape sensitivity to the modality of light appears to be rare. Although behavioral observations (Darwin, 1881; Hess, 1925; Nomura, 1926; Unteutsch, 1937; Howell, 1939), suggest that at least a few earthworm species withdraw their anterior ends when stimulated by sudden changes in background illumination, the possibility that such reactions may be mediated by giant nerve fibers has not been studied.

In this study we provide the first correlated electrophysiological and behavioral description of a rapid escape response to photic stimulation in an oligochaete worm. The worm, *Lumbriculus variegatus*, lives in the shallow margins of ponds, and conspicuously positions its tail segments at the air-water interface to facilitate gas exchange via the dorsal blood vessel. We show that the worm's tail is rapidly withdrawn in response to an abrupt decrease in light intensity or moving shadow, that sensitivity to such stimuli is restricted to posterior segments, and that such tail responses appear to be mediated by the lateral giant nerve fiber system. We have used electrophysiological and video recordings *in situ* to characterize the timing of electrical and behavioral events during shadow-evoked escape, and have compared these to touch-evoked responses. In addition, we have identified

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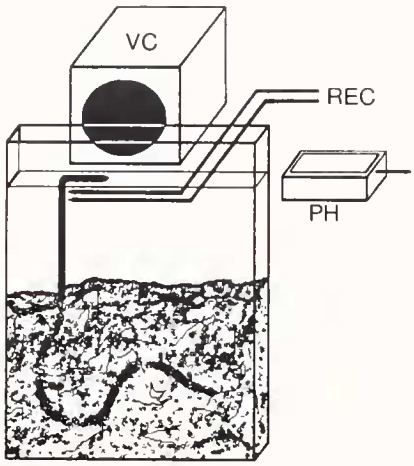

Figure 1. Test chamber for recording electrical and behavioral correlates of rapid escape in intact worms. The worm's tail protruded above the sediments and laid horizontally at the air-water interface. Recording electrodes (REC), submersed in the water and next to the tail, detected giant nerve fiber spikes while the video camera (VC) recorded escape movements. The photocell (PH) detected changes in the intensity of lighting from above.

candidate photoreceptor and mechanoreceptor cells in tail segments of the worm.

Materials and Methods

Animals

Freshly collected and fully grown specimens of *Lumbriculus variegatus* (Order Lumbriculida; Family Lumbriculidae) were used in all experiments. The average length of worms ranged from 5 to 7 cm and from 0.5 to 1.0 mm in diameter. The worms were collected during June–August from a pond adjacent to West Lake Okoboji (Gull Point), Iowa, and maintained as laboratory colonies in bowls containing debris (*i.e.*, leaf litter, wood fragments, and sediment) from the natural habitat. The water level was adjusted to approximately 3 cm above the debris, thus simulating typical habitat conditions. No aeration or supplemental food was provided.

Escape reflexes in intact worms

Worms were placed into individual glass-walled test chambers (75 mm high, 25 mm wide, 7 mm deep) containing debris from the natural habitat (Fig. 1). The water level was adjusted to 2–3 cm above the debris, and the chamber was placed on shock absorbing material.

Electrical recordings were obtained by mounting a pair of teflon-coated silver wire electrodes (0.01 inch diameter; 2 mm spacing between electrode tips) on a micromanipulator, immersing the electrodes into the water, and positioning the uninsulated electrode tips to within 1 mm of the worm's posterior end. Recorded activity was amplified (AC coupling; differential inputs), filtered, and displayed on an oscilloscope using a Tektronix 5D10 waveform digitizer. The digitized displays were either photographed on the oscilloscope screen or transferred, in analog form, from the digitizer to a chart recorder.

To study responses to a moving shadow, the test chamber was illuminated with four 15-W white fluorescent lamps placed 0.5 m above the water level. A moving shadow was created by interrupting the light path with a black shade attached to the end of a rigid arm. The arm was mounted on the rotating post of a pen driver motor driven by ramp voltages from a waveform generator.

Responses to abrupt decreases in light intensity were studied using a video camera (Panasonic Model WV-1400), fitted with a 100-mm camera lens and positioned at the side of the chamber. In addition to the main lighting previously described, a weakly lit white backdrop was positioned behind the chamber, providing sufficient contrast for videotaping escape movements even without the main lighting. The intensity of this lighting combination was 90-ft candles at the level of the water surface. The test stimulus was delivered by remotely switching off power to the main lighting, causing an abrupt decrease in light intensity to 6-ft candles at the water level. Stimulus onset was detected with a photocell, and interstimulus intervals were 30 min. All tests were at room temperature (22–24°C).

Escape responses in isolated body fragments

Since asexual reproduction by fragmentation is common in this species, most body fragments survive and regenerate into complete worms within several weeks. Body fragments ranging in length from 5 to 10 mm were obtained from anterior, middle, and tail regions using a dissecting scissors. All except 1 of 21 anterior fragments, 1 of 31 middle fragments, and 9 of 77 tail fragments survived at least 24 h after cutting.

Preliminary screening for behavioral responses to moving shadows was done between 8 and 12 h after cutting. Each fragment was placed into a flat glass dish containing a thin layer of water. The dishes were covered with a glass plate and illuminated as previously described. At 30-min intervals, a hand-held black screen was passed over each dish. Three replicate tests were done for each fragment. Escape responses to such shadow stimuli were clearcut; fragments either showed a vigorous shortening response or no overt response occurred.

Fragments that responded in preliminary screening tests were then used for studying the critical rate of shadow movement required for escape behavior, and the

responsiveness to mechanosensory stimuli or abrupt decreases in light intensity. Electrical recording were made as described for intact worms, but light conditions for videotaping behavioral responses were modified by positioning backdrop illumination below the flat dishes and reflecting the image of the fragment at a right angle into the video camera. Thus neither the mirror nor the camera interfered with the path of the main lighting from above. Mechanosensory stimuli to the fragments were delivered with the rounded head of an insect pin that was glued to the diaphragm of a small speaker. The speaker was mounted on a micromanipulator and driven by 1-ms pulses from a square pulse generator.

Microscopy

Fragments (5–10 segments long) were obtained from the tail and mid-body regions of worms. Fragments were fixed in 100-m*M* sodium cacodylate solution (pH 7.2) containing 2.5% glutaraldehyde and postfixed in osmium. The tissue was then dehydrated in an alcohol-propylene oxide series, and embedded in epon-araldite plastic. Cross-sections (1-μm thick) were cut, stained with 0.5% toluidine blue, and screened for candidate photoreceptor cells. Thin sections of those areas containing possible photosensitive regions were then prepared for transmission electron microscopy using slotted grids and uranyl acetate staining. For scanning electron microscopy, the tissues were fixed in 4% glutaraldehyde, dehydrated, and critically point dried for later viewing in a Hitachi S-800 field emission scanning electron microscope.

Results

Escape responses in intact worms

In many aquatic oligochaetes, including *Lumbriculus variegatus*, waveforms of all-or-none spikes from the medial (MGF) and lateral (LGF) giant nerve fibers are highly stereotyped and readily detected by non-invasive (transcutaneous) electrophysiological recordings using printed circuit board recording grids (Zoran and Drewes, 1987, 1988). The following are reliable diagnostic criteria for identifying LGF spikes and distinguishing them from MGF spikes in such recordings: (a) the LGF sensory field for mechanosensory stimuli includes the posterior two-thirds of the body, while the MGF field includes slightly more than the anterior one-third; thus there is only a small region of sensory field overlap; (b) LGF spikes are diphasic and often twice the amplitude and duration of the monophasic MGF spikes; (c) a single LGF spike is not usually followed by any detectable electrical potential from muscle, although two closely spaced LGF spikes often evoke a large muscle potential; in contrast, a single MGF spike is invariably followed after a few milliseconds by a small muscle potential; and (d) LGF conduction velocity is much slower than MGF velocity in the anterior and middle regions of the body.

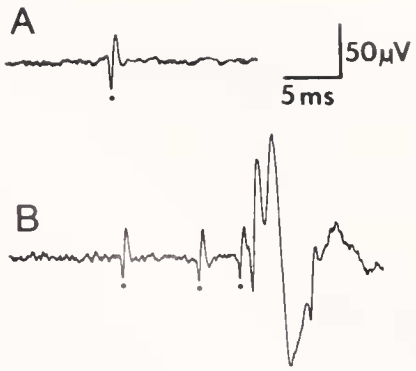

Figure 2. LGF spikes from intact worms. (A) One LGF spike (dot) was evoked by very light touch near the tip of the tail. (B) Repeated LGF spiking (dots) was evoked by a shadow moving across the worm's tail. The response was accompanied by a vigorous escape withdrawal of the tail.

To study the efficacy of mechanosensory and photic stimuli in evoking escape responses within a behavioral context, worms were placed individually into test chambers (Fig. 1). Within a few hours the worms had burrowed their anterior ends into the debris and projected their posterior ends vertically toward the water surface. Once the tip of the tail reached the surface, the terminal 15–30 segments of the tail became flexed ventrally, at a right angle, so that the dorsal surface of the body wall in these segments laid horizontally just above the surface of the water. In this position, the dorsal blood vessel, which lies just beneath the epidermis, began high-frequency, anterograde pumping of blood. Unless disturbed, worms remained in this stereotyped position for up to several hours, only occasionally readjusting their position.

A pair of recording electrodes was then very gradually positioned to less than 1 mm from the body wall of the projecting tail. LGF spikes, identical in waveform and other criteria described above, were readily detected in response to abrupt water displacement, direct touch to any portion of the tail, a moving shadow, or abrupt decrease in light intensity. Occasionally, only a single LGF spike was evoked in response to very light touch or moving shadow (Fig. 2A); however, such responses were insufficient to produce any detectable muscle electrical response or movement and, therefore, appeared to be subthreshold for overt behavior. Most commonly, however, these stimuli evoked two or more closely spaced LGF spikes (Fig. 2B) that were followed by larger, slower potentials (presumably from longitudinal muscle) and rapid escape shortening of the tail. Onset of these larger

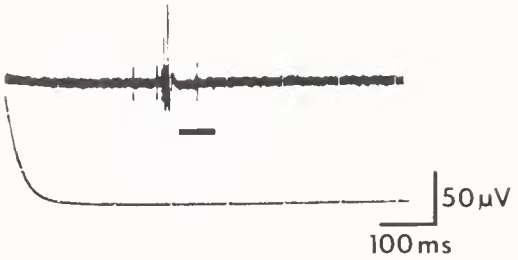

Figure 3. LGF response to an abrupt decrease in light intensity. In the upper trace, two LGF spikes (at least) preceded a burst of presumed muscle activity. The short bar below the trace designates when behavioral shortening occurred, as determined from videotape recordings. The lower trace shows the decreased output from the photocell, corresponding to onset of the stimulus.

potentials usually made it difficult to resolve any later LGF spiking in the response.

An example of LGF spiking in response to an abrupt decrease in light intensity is shown in Figure 3. Such responses were always accompanied by rapid escape shortening. The mean latency from stimulus onset to the first LGF spike was 314 ms ± 48 SD (n = 24 measurements; 11 worms).

An example of a videotape sequence of rapid escape withdrawal in response to an abrupt decrease in light intensity is shown in Figure 4. Here, marked tail withdrawal occurred between the ninth to eleventh frames after the stimulus onset. The mean latency from stimulus onset to detectable tail withdrawal was 389 ms ± 99 SD (58 measurements; 15 animals).

Animals were also tested for responsiveness to moving shadows or decreased light intensity at times when their tails were protruded vertically above the sediments, but not lying horizontally at the air-water interface. Care was taken to ensure that sufficient time had elapsed (>30 min) for recovery from previous test stimuli. Nevertheless, no escape withdrawal was observed in response to such stimuli, suggesting that the worm's responsiveness to moving shadow or abruptly decreased light intensity is dependent on its behavioral state.

Escape responses in body fragments

Isolated body fragments were screened for behavioral responsiveness to a moving shadow. The results (Fig. 5) showed that nearly half of the tail fragments, but no anterior or middle fragments, rapidly shortened in response to at least one of three test stimuli delivered at 30 min intervals. Failure of some test stimuli to evoke responses in otherwise responsive fragments did not appear to result from insufficient recovery time. Rather, the lack of responsiveness appeared to be related to variations in the behavioral state of the fragment. For example, we noted that if tail fragments were quiescent just before testing, then rapid escape responses were often evoked. However, if fragments were crawling or wriggling just before testing, then no rapid escape responses were evoked. These observations suggest that responsiveness to shadow may be reduced during such movements.

Next, electrical correlates of rapid escape movements were examined in tail fragments exhibiting shadow responsiveness. A very light touch stimulus anywhere on the fragment evoked one or more LGF spikes. As in intact worms, a single spike was never accompanied by a detectable muscle electrical response or movement (Fig.

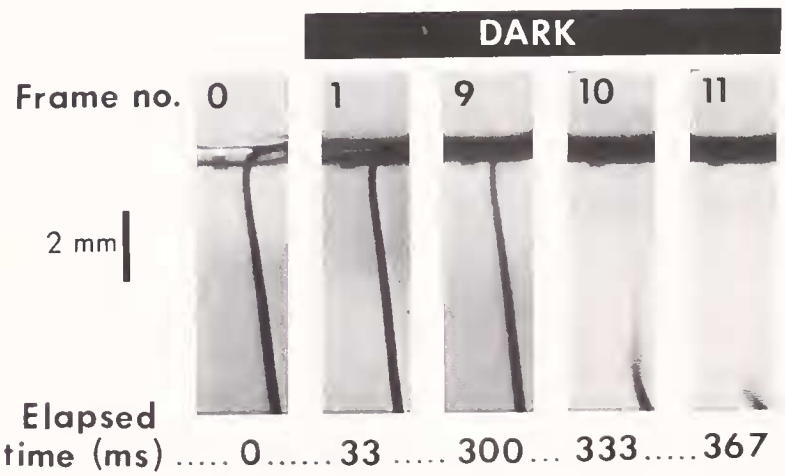

Figure 4. Frame-by-frame analysis of an escape response to an abrupt decrease in light intensity. Frame 0 shows the worm's tail lying at the air-water interface before onset of the stimulus. By the next frame (1) the lighting from above had been switched off, and between frames 9–11 the worm's tail rapidly withdrew from the water surface.

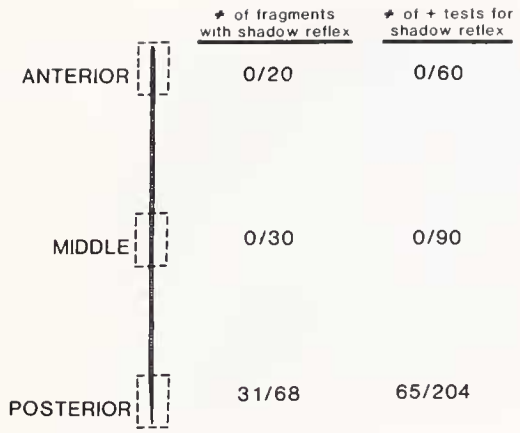

Figure 5. Results from behavioral screening tests with isolated fragments from anterior, middle, and posterior locations.

6A), but two or more closely spaced spikes usually evoked such responses (Fig. 6B). The latency from a touch stimulus to the first LGF spike ranged from 3–12 ms.

A moving shadow or abrupt decrease in light intensity also reliably evoked multiple LGF firing and muscle responses (Fig. 6C), resembling those obtained in intact worms. The minimal rate of shadow movement required for reliably evoking escape shortening was determined by passing a shadow at varying speeds over the tail fragments. The mean minimal rate was 4.4 mm/s ± 2.1 SD (n = 7 worms). This value appeared unaffected by changes in the orientation of the shadow relative to the longitudinal axis of the exposed tail.

The latencies of electrical and behavioral responses to abrupt decreases in light intensity were also determined, as described for intact worms. The mean latency from stimulus onset to the first LGF spike was 375 ms ± 96 SD (n = 23 measurements; 13 worms). A videotaped sequence of the behavioral response to the same stimulus is shown in Figure 7. The mean latency to the onset of such responses was 449 ms ± 67 SD (n = 24 measurements; 9 worms). Thus, although latencies to electrical and behavioral responses were somewhat longer in tail fragments than intact worms, the same general pattern of LGF responsiveness to photic stimulation was seen in both tail fragments and intact worms.

Since results from isolated tail fragments suggested that the sensitivity of escape responses to shadow was localized in tail segments, these fragments were examined for candidate photoreceptor cells.

Candidate photo- and mechanoreceptors in posterior fragments

Since physiological and behavioral results from isolated body fragments suggested that tail fragments were particularly responsive to shadow (as well as touch) these fragments were microscopically examined for candidate photoreceptors and mechanoreceptor cells.

Light microscopy and scanning electron microscopy revealed no obvious ocellar-like structures on the cuticular surface, although many complex arrangements of long and short ciliary processes, intermingled with large numbers of short microvillar projections, were evident on the cuticular surface (Fig. 8A). However, transmission electron microscopy of the epithelial surface revealed candidate photoreceptor cells. These were similar to the simple phaosomal-type photoreceptors described in the anterior segments of leeches and earthworms (reviews by Sawyer, 1986, and Jamieson, 1981). These cells were located on the dorsal surface of the tail and were sparsely distributed. Usually two, but occasionally as many as four, were found per segment. Cursory observations in mid-body segments of three animals revealed no evidence of comparable cells.

Figure 8B–C shows the candidate photoreceptors from two different worms. The cells have a large apical phaosome (varying in size from 1.5 to 4.0 μm) with a broad opening to the external cuticle. The phaosome contains numerous microvilli (0.10 to 0.15 μm in diameter), most projecting centrally into the phaosome cavity and a few projecting through the phaosomal opening and into the cuticular layer. Other features of these cells include: (1) extensive tight junctions with surrounding epithelial

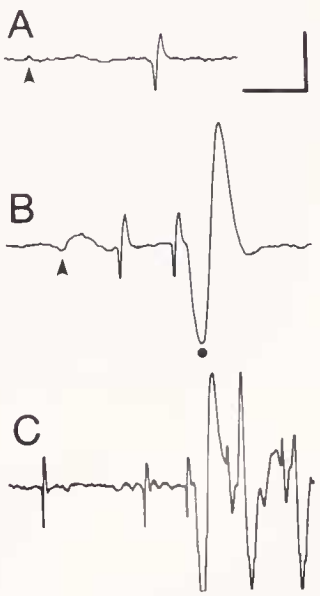

Figure 6. LGF responses in isolated tail fragments. (A) One LGF spike was evoked by a mechanical stimulus (arrow). (B) Two LGF spikes, followed by a presumed longitudinal muscle potential (dot), were evoked by another, slightly stronger mechanical stimulus. (C) A train of LGF spikes and muscle response were evoked by a moving shadow. Time scale: 5 ms (A, B); 10 ms (C). Voltage scale: 50 μV.

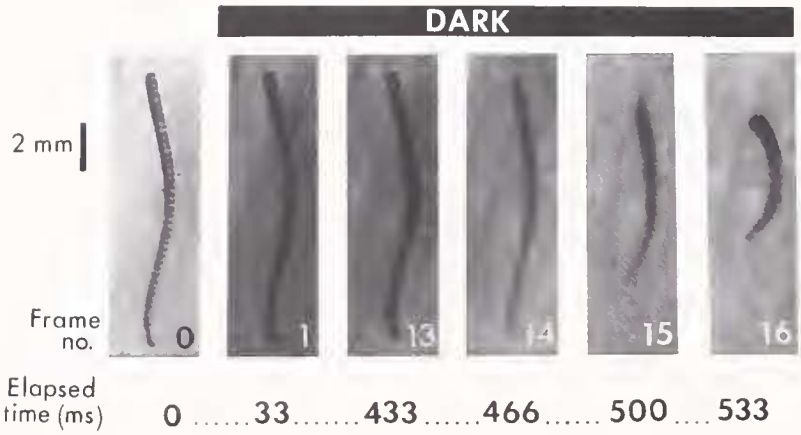

Figure 7. Frame-by-frame videotape sequence of rapid shortening in an isolated tail fragment in response to an abrupt decrease in light intensity. Frame 0 shows the tail fragment before onset of the stimulus. Rapid shortening occurred between frames 13 to 16.

cells (Fig. 8C); (2) a basal nucleus; and (3) basal projections into a radial nerve plexus directly beneath the epithelial layer.

Thin sections through the body wall also provided details regarding the ciliated epithelial cells (Fig. 8D, E). Both uniciliate and multiciliate cells were evident. The multiciliate cells generally have four to six cilia with a corresponding number of basal bodies. In the uniciliate cells, the cilium arises from a pit in the apical region of the cell and is surrounded by 10 microvillar projections, each containing an abundance of microfilaments and an electron-dense region adjacent to the ciliary process. The microvilli (approximately 0.3 μm in cross-sectional area and 0.15 μm in width) are also interconnected via an organized, electron-dense material (Fig. 8E). These two ciliated cell types are nearly identical to the proposed epithelial mechanoreceptors described in the anterior segments of earthworms (Knapp and Mill, 1971; Mill, 1982) and an aquatic oligochaete, *Rhynchelmis limosella* (Moritz and Storch, 1971), the latter representing the same family as *Lumbriculus (i.e.,* Lumbriculidae).

Discussion

Adaptive significance and timing of the shadow reflex

Stimulus modalities that elicit rapid tail withdrawal in *L. variegatus* include touch, moving shadow, and abrupt decrease in light intensity. These modalities, as well as the specific escape movements they elicit, appear well matched to the worm's specific lifestyle and habitat. The worms are especially abundant in the shallow margins of ponds where their tails protrude several centimeters above the sediments and lie horizontally at the air-water interface (Fig. 1), a position that apparently facilitates gas exchange via the dorsal blood vessel. In this position, the exposed tail would be vulnerable to attack by subsurface, surface, or aerial predators.

Because pond water is often highly turbid, and candidate photoreceptor cells in exposed segments tend to be located in a dorsolateral position, mechanosensory, rather than photosensory, cues may be more important in the detection of subsurface predators (*e.g.,* aquatic insects or fish). On the other hand, photosensory cues may be important in signalling the approach of surface or aerial predators (*e.g.,* amphibians or birds). This idea is consistent with observations that moving shadows above the water surface reliably elicit rapid tail withdrawal, either in small laboratory aquaria containing natural sediments (Fig. 4), or in actual field settings (C. Drewes, unpub.).

Although the electrophysiological and behavioral events during responses to photic stimuli appeared indistinguishable from those elicited by mechanosensory stimuli, the timing of escape responses to these two stimulus types differed markedly with respect to onset latency. The latency between a mechanical stimulus and the onset of LGF spiking was usually less than 12 ms, a value comparable to those in other terrestrial and aquatic oligochaetes (Drewes, 1984; Zoran and Drewes, 1987). In contrast, latencies following a photic stimulus were much longer, ranging from 250–375 ms. Such values are similar to those seen in other annelid escape responses to photosensory stimuli, such as giant fiber responses to shadow in polychaetes (Gwilliam, 1969) and S-cell responses to light flashes in leech (Laverack, 1969; Bagnoli *et al.,* 1973).

Several possible factors could contribute to these relatively long latency values. (1) The response time of the photoreceptors may be relatively slow. For example, in leech photoreceptor cells, the time from onset of a photic

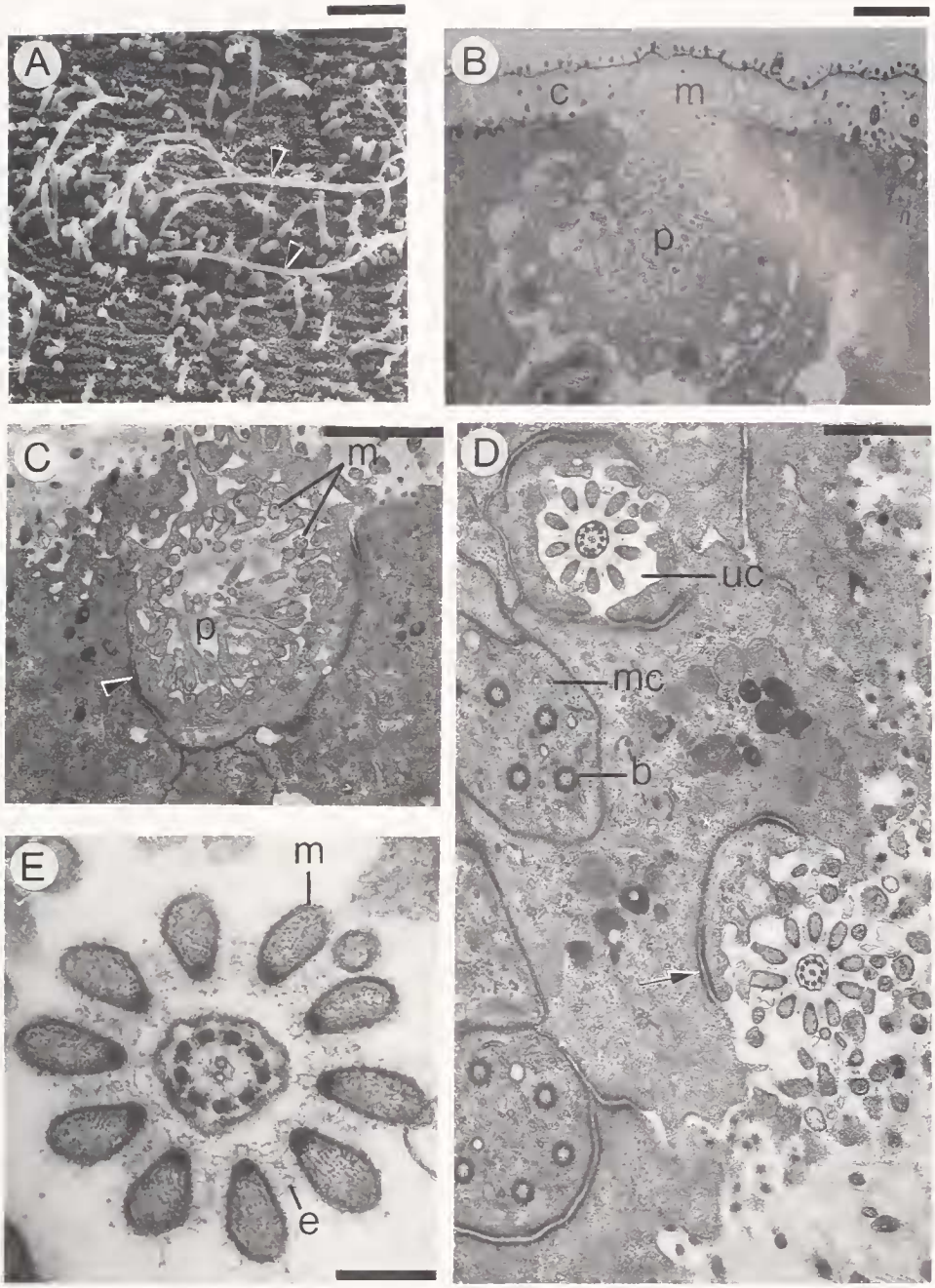

Figure 8. Scanning and transmission microscopy of body wall of the tail of *L. variegatus*. The scanning image (A) reveals a rough cuticular surface and an abundance of microvilli and cilia protruding from the surface. Note the long cilia (arrows) lying parallel to the surface. The cross-section (B) and frontal section (C) through the phaosomal region of the presumed photoreceptor reveals numerous microvilli (m) projecting into the phaosome cavity (p) and cuticle (c). The presumed photoreceptors are linked to adjacent epithelial cells via tight junctions (arrows in C and D). A frontal section (D) of the body wall shows uniciliary (uc) and multiciliary cells (mc). Note the basal bodies (b) of the multiciliary cell. A cross-section (E) of the cilium from a uniciliary cell reveals the standard 9 + 2 cilium surrounded by ten microvillar processes. Adjacent microvilli are connected via a circular complex of extracellular fibers (e). Scale bars: A, C, 2.0 μm; B, D, 1.0 μm; E, 0.25 μm.

stimulus to the first spike in the photoreceptor cells is approximately 80 ms (Lasansky and Fuortes, 1969; Fioravanti and Fuortes, 1972; Peterson, 1984). (2) Conduction and synaptic transmission from the peripheral photoreceptors to through-conducting fibers in the central nervous system may be time-consuming, especially if

pathways are small in caliber and include interposed sensory interneurons, as proposed in the leech (Kretz *et al.*, 1976). (3) Additional time may be consumed for spatial integration and parallel processing of segmental inputs onto giant fibers.

These factors, although detrimental in terms of increasing response time, may be advantageous by providing an opportunity for appropriate modulation of escape response sensitivity. For example, our results suggest that escape response sensitivity depends on the worm's behavioral state, with tail segments being especially responsive to shadows when positioned horizontally at the air-water interface. This suggests that specific combinations of sensory cues may somehow interact to modulate escape responsiveness, a phenomenon that has been experimentally demonstrated for converging photosensory and mechanosensory inputs onto S-cell interneurons in the leech (Bagnoli *et al.*, 1973).

Candidate photoreceptor cells

Photoreception in oligochaete worms is predominantly mediated by extraocular, dermal photoreceptors (Steven, 1963; Yoshida, 1979; Welsch *et al.*, 1984). Evidence from two lumbricid earthworm species, *Lumbricus terrestris* and *Eisenia foetida*, indicate that the photoreceptor cells have a characteristic microvillar organization. That is, the receptor cell contains a large, membrane-bound cavity (termed the "phaosome") which is lined by microvilli (review by Jamieson, 1981). Our results (Fig. 8) indicate that this same type of receptor cell exists in the epidermis of tail segments in the aquatic oligochaete, *L. variegatus*. Because isolated tail fragments are capable of reacting to decreased light intensity (Fig. 5), and because we have been unable to find other candidate photoreceptor cells in these fragments, we infer that the phaosomal photoreceptors somehow mediate the worms' reactions to these stimuli.

The ultrastructural organization of these epidermal photoreceptor cells closely resembles that of the earthworm, *Eisenia foetida*, in having a relatively apical position in the epidermis, a free space in the center of the phaosome, and a microvillar-lined canal, which joins the phaosomal cavity with the extracellular space beneath the body wall cuticle (Hirata *et al.*, 1969). These features are also evident in photoreceptors of the medicinal leech (review by Sawyer, 1986) but not in the common earthworm, *Lumbricus terrestris*. In the latter case, the microvillar photoreceptor cells are located in the basal portion of the epidermis and the phaosome does not open to the outside (Rohlich *et al.*, 1970; Myhrberg, 1979).

Based on microelectrode studies of leech photoreceptor cells (Lasansky and Fuortes, 1969; Fiorvanti and Fuortes, 1972; Peterson, 1984), an abrupt increase in light intensity causes a large depolarizing receptor potential and marked increase in action potential firing in the cell. Since the adequate stimulus for eliciting an escape response in *L. variegatus* is an abrupt decrease in intensity, an interesting question arises as to the polarity of its photoreceptor electrical response. If the polarity is identical to that in the leech, then a shadow stimulus would be expected to hyperpolarize the receptor cell and excitation of the LGF system would therefore necessitate a step of disinhibition somewhere along the afferent pathway. Progress in resolving this question will require developing a reliable means of identifying and recording from the photoreceptor cells in dissected preparations. This may be difficult in view of the relatively sparse distribution of receptors cells in the epidermis and the proclivity for segmental autotomy when attempting to dissect this species. On the other hand, the associated high capacity for segmental regeneration by body fragments, in combination with a segmental respecification (morphallaxis) during regeneration (Drewes and Fourtner, in prep.), may offer unusual opportunities for investigating behavioral, anatomical, and physiological correlates of developmental plasticity in the shadow reflex of this species.

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